Interdepartmental Executive Committee on Pest Management
Le Comité interministériel exécutif sur la lutte antiparasitaire

Regulatory Directive **Dir94-08**

Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits

This document replaces Regulatory Proposal 94-01

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Part A - Introduction

A1.0 Preamble

Since 1988 Agriculture and Agri-Food Canada (AAFC) has been regulating the field testing in Canada of agricultural and horticultural crop plants with novel traits (PNT's).

A Plant with Novel Traits is defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change.

Familiarity is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. Substantial equivalence is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

This testing includes products of both recombinant DNA technology and plants derived through traditional plant breeding. Regulated field testing is necessary when the PNT's have traits of potential concern, i.e., the traits themselves, their presence in a particular plant species or their use are: (1) considered unfamiliar when compared with products already in the market; (2) not considered substantially equivalent to similar, familiar plant types of the same species, in use, and already regarded as safe. Applicants are encouraged to review Figure 1, in Appendix I, which will assist in deciding whether their plant type triggers an environmental safety process.

Regulated trials have been authorized to be conducted under conditions of confinement[1], such as reproductive isolation, restrictions on post-harvest land use, and disposal of seed and plant residues. In addition, sites are inspected by AAFC personnel both during and following the trials.

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[1] Applications to conduct a confined field test of a PNT are evaluated and authorized by the Plant Products Division of the Plant Industry Directorate (PID). All applications to conduct these tests are also evaluated by the Plant Health Risk Assessment Unit of the Research Division of the Animal and Plant Health Directorate (APHD), on behalf of the Plant Protection Division of APHD, for potential plant health risks. The testing of a PNT may also involve the use of a pesticide; such use falls under the authority of the *Pest Control Products (PCP) Act*. Procedures are in place to ensure that the Product Management Division of PID is informed whenever a pesticide-tolerant plant is tested. Applicants must meet the requirements of the *PCP Act*. Under conditions of confinement, PNT's field tested to date include canola, rapeseed, flax, soybean, corn, tobacco, alfalfa, potato, wheat, strawberries and tomato. Please refer to the latest version of the Directive *Field Testing Plants with Novel Traits (or Genetically Modified Plants) in Canada: Guidelines and Information Required for Submitting an Application for Field Trials Under Confined Conditions*, available from the Plant Products Division.

The authorizations have taken into consideration potential risks and, depending on these risks, applicants are required to take steps to mitigate potential problems.

Regulated trials will continue but some PNT's will be eventually considered for open or unconfined release into the environment, towards the time of commercialization. Before PNT's may by authorized for unconfined release they must be assessed for environmental safety. This document addresses the information necessary to identify potential adverse environmental impacts associated with the unconfined release of the PNT, including the end use of the PNT. This is in keeping with the *Canadian Environmental Protection Act (CEPA),* and amendments to the *Seeds Regulations* (Part V). Specific information requirements will vary with the species, characteristics of the novel trait and the PNT's end use. The information must address three issues:

- identity of the PNT,
- relative phenotypic expression, and
- potential interactions of the PNT with other life forms.

It should be noted that this is a federal regulatory document. The appropriate provincial regulatory authorities are being consulted to ensure a "single window" approach and harmonization of regulations.

A2.0 Scope and Purpose

A2.1 The scope of this document covers all plants with novel traits that are of concern (i.e., that are not familiar or substantially equivalent to products already on the market, in use and generally regarded as safe).

A2.2 The purpose of this document is to define criteria and information requirements that must be considered in the environmental assessment of PNT's to ensure safety, in the absence of confinement conditions. This includes identification of potential concerns, required information, and procedures to assess potential environmental impacts associated with unconfined release of PNT's. The assessment criteria are designed to be used in conjunction with species-specific companion documents, that describe the biology of the species to which the modified plant belongs, including details of other life forms with which it interacts. The assessment will be part of the continuum of research, development, evaluation and commercialization of plants with novel traits (Appendix I, Figure 2).

A2.3 Assessment criteria: Unconfined release consists of use, without requirements of reproductive isolation, post-harvest land use and monitoring by AAFC. The environmental effects that will be assessed prior to release include:

- potential of the PNT to become a weed[2] of agriculture or be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest[3],
- potential impact of the PNT or its gene products on non-target species, including humans,
- potential impact on biodiversity[4].

A2.4 Due to the broad range of PNT's that are being developed, this document and its guidelines should be considered flexible and will likely evolve as more experience is gained. A determination of the need for unconfined release authorizations and the environmental safety assessment of PNT's will be conducted on a case-by-case basis, founded on familiarity and substantial equivalence.

A3.0 Definitions

A3.1 **Biodiversity:** The variety of life and its processes. Biodiversity includes all life forms, from one-celled fungi, protozoa and bacteria to complex organisms such as plants, insects, fishes and mammals. It includes processes, pathways and cycles that link living organisms into populations, ecosystems and landscapes. This variety of life is dynamic and constantly changing and evolving. It is sensitive to perturbations that may result from human activity. Biodiversity is generally recognized on three levels:

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2 For the purpose of this document, weeds are considered a subset of plants that may be considered pests. The term weed is used to describe a plant species that is a nuisance to humankind in that it occurs in "managed" ecosystems where it is unwanted. Weeds tend to spread easily in disturbed areas or among crops. Whereas it can be considered that any "plant out of place" is a weed, the emphasis of these guidelines is to determine whether a PNT could be successful in colonizing managed ecosystems at the expense of displacing other species, in particular cultivated crop plants. Control of very weedy plants can be both financially and environmentally costly.

Should a PNT become successful in colonizing natural "un-managed" ecosystems, and in the process displace other species and disrupt those ecosystems, for the purpose of this document the species would be considered invasive of such natural ecosystems.

3 A plant may be a pest without necessarily being considered weedy. For instance, a pest status could result from the production of allergenic or toxic substances.

4 Given the complexity of biodiversity, the assessment of environmental impact of PNT's can only be completed indirectly.

- genetic diversity - the variety of genetic building blocks found among individual representatives of a species;
- species diversity - the variety of living organisms found in a particular place; and
- ecosystem diversity - the variety of species and ecological functions and processes, both their kind and number, that occur in different physical settings.

A3.2 Confined Field Testing: It involves the release of a PNT under terms and conditions of confinement including reproductive isolation, site-monitoring and post-harvest land use restriction. Within these conditions, trials designed to determine outcrossing rates in outcrossing species, "weediness" characteristics of the PNT, persistence in the environment, impact on other species and other studies may be permitted.

A3.3 Counterpart: The chosen counterpart should, if possible, be the unmodified host plant genotype (closest genetic equivalent) and may be a previously assessed and approved PNT. If it is not similar to the PNT (e.g., in the case of an F1 hybrid), the counterpart must be a similar genotype/phenotype of the same species. In the case of an F1 hybrid, consideration may also be given to comparing the parental lines with novel traits with similar counterparts. Since there will be a range of characteristics among varieties within a species, comparison with several counterparts may show that the PNT has characteristics within the normal range exhibited by that species.

A3.4 Environment: Components of the earth (including air, land, water, all layers of the atmosphere, all organic and inorganic matter and living organisms) and all interacting natural systems that include components referred to above *(CEPA, Section 3).* Includes the natural and managed ecosystems including agricultural ecosystems.

A3.5 Environmental Effect: Any significant change that the release of the PNT may cause in the environment.

A3.6 Environmental Risk: Risk is defined in terms of probability of the occurrence of an effect, multiplied by the hazard (i.e., the degree of harm that results from the effect).

A3.7 Familiarity: The knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada.

A3.8 Genotype: The genetic constitution of an organism.

A3.9 Phenotype: The observable properties of an organism, generally regarded as the product of the interaction of the organism's genotype with its environment.

A3.10 Plant Pest: Any plant that is injurious or troublesome, or whose products or by-products are noxious or troublesome.

A3.11 Plant with Novel Traits (PNT): A plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change.

A3.12 Substantial Equivalence: The equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

A3.13 Unconfined Release: The use of a PNT that is not subject to reproductive and physical isolation from the natural or agricultural environment, site inspections, post-harvest land use restrictions and/or restricted use of seed and progeny[5].

A4.0 Processing of Applications

Applications that are complete will allow for timely evaluations with a minimum of correspondence requesting further information.

Plant Products Division, as lead agency for the environmental safety assessment of PNT's, may distribute, within Agriculture and Agri-Food Canada, a copy of the application to:

- the Plant Health Risk Assessment Unit, Animal and Plant Health Directorate (APHD), on behalf of the Plant Protection Division, APHD;

- the Product Management Division, Plant Industry Directorate (PID), if the material involves altered pesticidal tolerance or altered pesticidal properties.

Evaluations from the secondary agencies are returned to the Plant Products Division, and a final evaluation performed, incorporating any comments. From this, a decision will be made whether or not an unconfined release should be authorized.

Prior to commercialization, depending on the use of the harvested product, Health Canada may require information allowing a determination of safety of the product as a novel food, and/or the Feed Section of the Plant Products Division may require information allowing a determination of safety of the product as a novel feed.

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[5] However: • for outcrossing species with certain specific traits, reproductive isolation may always be required;
• monitoring by the applicant will be required.

Both Health Canada and the Feed Section will be informed if the applicant has clearly requested these safety determinations. It is recommended that the developer of a PNT inform these agencies, as early as possible, of intent to commercialize such plant material.

Part B - How to Complete an Application for Determination of Environmental Safety of Plants with Novel Traits.

Applicants are encouraged to consult the Plant Products Division, as lead agency, in the early stages of the development of the PNT, in order to reach agreement on whether an environmental release authorization is required and, where appropriate, for clarification on what specific information is necessary for an assessment.

B1.0 Where to Apply

Please address your application for determination of environmental safety of PNT's to:

The Director
Plant Products Division
Agriculture and Agri-Food Canada
59 Camelot Drive
Nepean, Ontario
K1A 0Y9

Telephone: (613)952-8000

The assessment process will not begin unless **four copies of the application are submitted.** This is because of the notification of the application to other relevant federal government agencies, who may be asked to comment on general or specific parts.

B2.0 When to Apply

The developer of a PNT is strongly encouraged to apply for assessment of environmental safety well in advance of the anticipated time of commercialization. Applications for assessment of environmental safety will be processed on a first-come-first-served basis. Degree of completeness of the application will be a factor in the length of review period required.

B3.0 How to Apply - Completing an Application

Send a covering letter with your application, summarizing the request for assessment and an explanation as to why the PNT in question is not familiar nor substantially equivalent. Include in this summary a description of the plant species, the novel traits and the potential geographic scope of release.

Statements in support of a developer's application should clearly describe the test procedures followed in developing the test data, including test methods, reference products, quality control, quality assurances procedures, together with bibliographic references where these are appropriate. It is expected that other supporting information and test data, that are relevant to environmental and human health exposure and hazard identification and that are in the applicant's possession or to which the developer ought reasonably to have access, must be included in the application.

In completing the application, applicants may consider it unnecessary or inappropriate to provide certain information. In these instances, information requirements **may be waived if valid scientific rationale is provided. Please note that all the information in Part C must be provided.**

B4.0 Information Considered to be Confidential

Please indicate in the application which information is to remain confidential business information (CBI). However, please do not apply a confidential stamp to cover all pages of the application, unless you really consider all included information on a page to be confidential.

As a member of the Organization for Economic Cooperation and Development (OECD), Plant Products Division submits non-confidential information on environmental release of PNT's to their publicly available Biotrack database. It is therefore important that CBI be clearly identified.

Although all information indicated as CBI may be initially retained as confidential, the retention of this information as CBI is subject to the federal *Access to Information and Privacy (ATIP) Acts*. Please consult with AAFC's ATIP Service, at (613) 995-5118, for further information.

B5.0 Information Requirements for Determining Environmental Safety

Information requirements fall into three categories and a summary of anticipated effects:

- **information clearly showing the identity and origin of the PNT;**
- **information clearly identifying and describing properties of the novel gene products; and**
- **information determining the relative phenotypic expression of the PNT compared to a similar counterpart, where differences are anticipated.**

and, based on these

- **a summary of anticipated relative impacts resulting from the release.**

This information will be used to determine relative changes and impacts, and in addition, should be summarized in Tables 1-4 found in Appendix II. The relative changes and impacts will be compared to the biology of the counterpart of the PNT.

The biology of specific species is described in a series of species-specific companion documents, that will act as a guide to generating comparative data, where required. These documents will include information about the major interactions of the unmodified or normal plant species with other higher life forms (such as predators, grazers, parasites, pathogens, competitors and symbionts and beneficial organisms and including humans where appropriate) in the production range of the PNT, to help identify potential risks associated with the PNT relative to a counterpart of the same species. Applicants are expected to provide a similar description of the biology of the species if it is not available in the companion document series.

B6.0 More About the Tables

In addition to the information provided in text form, the following information should be included in the four Tables:

Table 1: Specific identity of the PNT including its novel traits and any genetic constructs or modifications introduced into the PNT under review. If the PNT is derived through conventional plant breeding, pedigree and selection information that confirms its origin must be given, including any relationship to a previously authorized PNT. The end use of the PNT must also be described. Certain information in this Table may be considered by the proponent to be CBI.

Table 2: Specific genetic modification, including novel gene products and their activity in the PNT and the environment. Certain information in this table may be considered by the proponent to be CBI. This information will be removed from any public database to protect intellectual property rights.

Table 3: Phenotypic expression of the PNT relative to a corresponding counterpart of the same species. Certain information in this Table may be considered by the proponent to be CBI[6].

Table 4: Summary of the potential consequences of the unconfined use of the PNT for both the natural and agricultural environments, and identification of potential impacts of this release. The column headings termed "Relative

[6] While certain information detailed in Tables 1, 2 and 3 may be considered confidential business information, the information supplied in Table 4 will be considered to be in the public domain, and will be available to interested parties upon request. Although all information indicated as CBI may be initially retained as confidential, the retention of this information as CBI is subject to the federal *Access to Information and Privacy (ATIP) Acts*. Please consult with AAFC's ATIP service at 613-995-5118, for further information.

Impact" are intended to be a measure of the relative merit or demerit of the effect of this release when considered across the columns of "Degree of Change", "Geographic Scope" and "Duration". The term "Agronomic Practices" indicates the effect of the environmental release on agronomic practices such as pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation.

B7.0 Measures Taken by AAFC Following the Environmental Assessment

Plant Products Division, in consultation with the Plant Health Risk Assessment Unit (on behalf of the Plant Protection Division) and the Product Management Division, will use the information provided by the applicant to determine if the PNT poses potential adverse environmental impacts.

B7.1 Based on this assessment, Plant Products Division will either (see footnote 5):

1) grant approval for the unconfined release of the PNT, or in the case of certain traits, release under specified conditions (eg., as with high erucic acid rapeseed, which must not enter canola marketing channels) and make this information available to other stakeholders, or

2) prohibit unconfined release of the PNT with the reasons for prohibition (again, this information will be made available), or

3) follow-up with the applicant for additional information that Plant Products Division deems necessary to complete the assessment, followed by decision 1) or 2) above.

B8.0 Post-Release Monitoring

Post-release monitoring must be carried out by the applicant. If, at any time after authorization for unconfined release, the applicant becomes aware of any new information regarding risks to the environment (e.g., enhanced weediness characteristics) or human health (e.g., exposure to allergens) that could be caused as a result of the release, the applicant must immediately inform the Director of the Plant Products Division.

Part C - Required Information for Determining Environmental Safety

Applicants should clearly describe the methods used to obtain the requested information together with bibliographic references, including numbered patents, where these are appropriate.

The following information is required:

C1.0 Personnel Involved and Status of the PNT in the Application

C1.1 Applicant:
1) Name
2) Address
3) Telephone Number
4) Facsimile Number

C1.2 Canadian representative, if different from above:
1) Name
2) Address
3) Telephone Number
4) Facsimile Number

C1.3 Is the plant material imported? If yes, was an import permit applied for under the *Plant Protection Act*? Was it granted? If yes, provide the permit number if known.

C1.4 Was the plant material previously tested in Canada? If yes, in what years?

C1.5 If the PNT was derived through recombinant DNA techniques, were the gene constructs previously tested in Canada? If yes, in what plant species and in what years?

C1.6 Were other government agencies, either foreign or within Canada, notified of the development of the PNT or its importation? What was the purpose of such notification?

C2.0 Description of the PNT and its Modification

Use Appendix II, Table 1 to summarize this information, where appropriate. Applicants who have previously applied for, and received, authorization to field test PNT's using the latest version of the Directive *Field Testing Plants with Novel Traits (or Genetically Modified Plants) in Canada: Guidelines and Information Required for Submitting an Application for Field Trials Under Confined Conditions* may refer back to information supplied in Part C, section IV "Description of the Modification" and section V "Modified Plant Material", of that Directive. Please ensure that any questions not previously addressed, are answered in this safety assessment document.

C2.1 Describe the following about the PNT:

1) Confirmation of taxonomy;

2) Designation given to the PNT, including all synonyms;

3) Pedigree information of the PNT (including any relationship to a previously assessed PNT);

4) Give details of the use of the PNT (e.g., to be grown as a field crop for grain production; to be grown as field crop for grain production on lands contaminated with persistent herbicide; to reclaim lands contaminated with heavy metals).

C2.2 Describe the following about the modification:

1) Novel gene products conferring the novel traits.

2) Methods used to introduce the novel traits (briefly describe the techniques, if not through recombinant DNA).

3) If the modification was achieved through recombinant DNA techniques:

a) supply a map of each genetic construct.
b) for each genetic construct, list, identify source and describe in detail:
   i) genes, including antibiotic resistance, other marker genes or regulatory genes;
   ii) the products of the introduced genes;
   iii) regulatory sequences, i.e., promoters, modifiers, enhancers, signal peptides, and terminators;
   iv) any other DNA sequences.
c) was the transformation vectorless? If yes, describe in detail.

d) if a vector was employed, answer:
   i) what is the vector name and cloning method?
   ii) is the vector naturally pathogenic?
   iii) was the vector disarmed?
   iv) how was the vector disarmed?
   v) is there expression of the gene in the vector?

C2.3 In the case of an allopolyploid PNT, in which parental genome is the genetic modification?

C2.4 Number of generations removed from the original modification.

C2.5 Once inserted into the plant, has each genetic modification and its expression been shown to be stable? Provide data demonstrating stability.

C3.0 Description of the Novel Traits

This information should be summarized in Appendix II, Table 2 and will identify areas of potential interactions of the PNT with the environment. Differences in these interactions from the original unmodified plant or counterpart will be compared in Part D.

C3.1 Characterize in detail the gene products, breakdown products, by-products and their metabolic pathways.

C3.2 Are the gene products tissue-specific?

C3.3 Are the genes expressed during a specific developmental stage?

C3.4 Is gene expression induced? If yes, what are the inducing agents?

C3.5 Describe the activity of the gene products, breakdown products and by-products in the host plant. Describe any changes to existing metabolic pathways (including altered accumulation and storage patterns), including those that might not be intended.

C3.6 The toxicity of the novel gene products, breakdown products and by-products in the environment must be established. Describe:

1) potential toxigenicity to known or potential predators, grazers, parasites, pathogens, competitors and symbiont;

2) potential for adverse human health effects, e.g., exposure to toxins, irritants and antigens. Include estimated level and most likely route of human exposure to the gene products, breakdown products and by-products.

Part D - Biology and Interactions of the PNT

This information is intended to permit a determination and identification of anticipated or observed differences between the PNT and the unmodified form or a named counterpart (closely related genotype, or with a range of named counterparts of similar plant type. See B5.0). **Specifically, this is to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats or be otherwise harmful to the environment.**

D1.0 Interactions of the PNT

Information in this section should be used to complete Appendix II, Tables 3 and 4 (again, as required in Part C, applicants should clearly describe the methods used to obtain the information together with bibliographic references, including numbered patents, where these are appropriate. In addition, data should be obtained from experimental designs using sound statistical methods, where appropriate). **In completing the tables, applicants may consider it unnecessary or inappropriate to provide certain information. In these instances, information requirements may be waived if valid scientific rationale is provided.**

D1.1 Relative phenotype expression of the PNT (species replacement or competition studies may be appropriate when there is reason to believe that the biology of the plant has been altered in unpredictable ways. See Appendix III):

1) Reproductive and survival biology;

2) Adaptations to stress factors (for biotic stress factors, identify those life forms with which the PNT interacts differently);

3) Biochemistry: For novel gene products identified in Part C3.6 that are known to be toxic, describe:

   a) likelihood and change of level of exposure of consumers and symbiont;
   b) the effect on soil micro flora and fauna. Residual studies may be conducted to determine macro changes. Observed changes at this level may warrant further in-depth studies (see Appendix IV).

D1.2 Agricultural-Silvicultural Practices:

1) What are the proposed release sites for the PNT?

   a) all of Canada?
   b) specific regions?
   c) what is the projected area (ha) of release?

2) Will the modification result in the PNT being grown outside of the normal geographic production area for the species?

3) Will the modification result in the PNT being grown outside of the usual habitat (e.g., cultivated agricultural lands) for the species?

4) Will the cultivation practices (land preparation, weed and pest control, harvest, and post-harvest protocols) involved in growing the PNT vary

from those traditionally used?

a) if no: no further information required
b) if yes:
   i) describe the change in cultivation practices;
   ii) provide information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation;
c) will volunteer plants of the PNT result in altered cultivation practices for succeeding crops?

5) If it is anticipated that the PNT will be cultivated only under contract/controlled conditions, describe:

   a) any control and mitigation procedures;
   b) post-harvest procedures, including procedures for disposal of remaining plant matter.

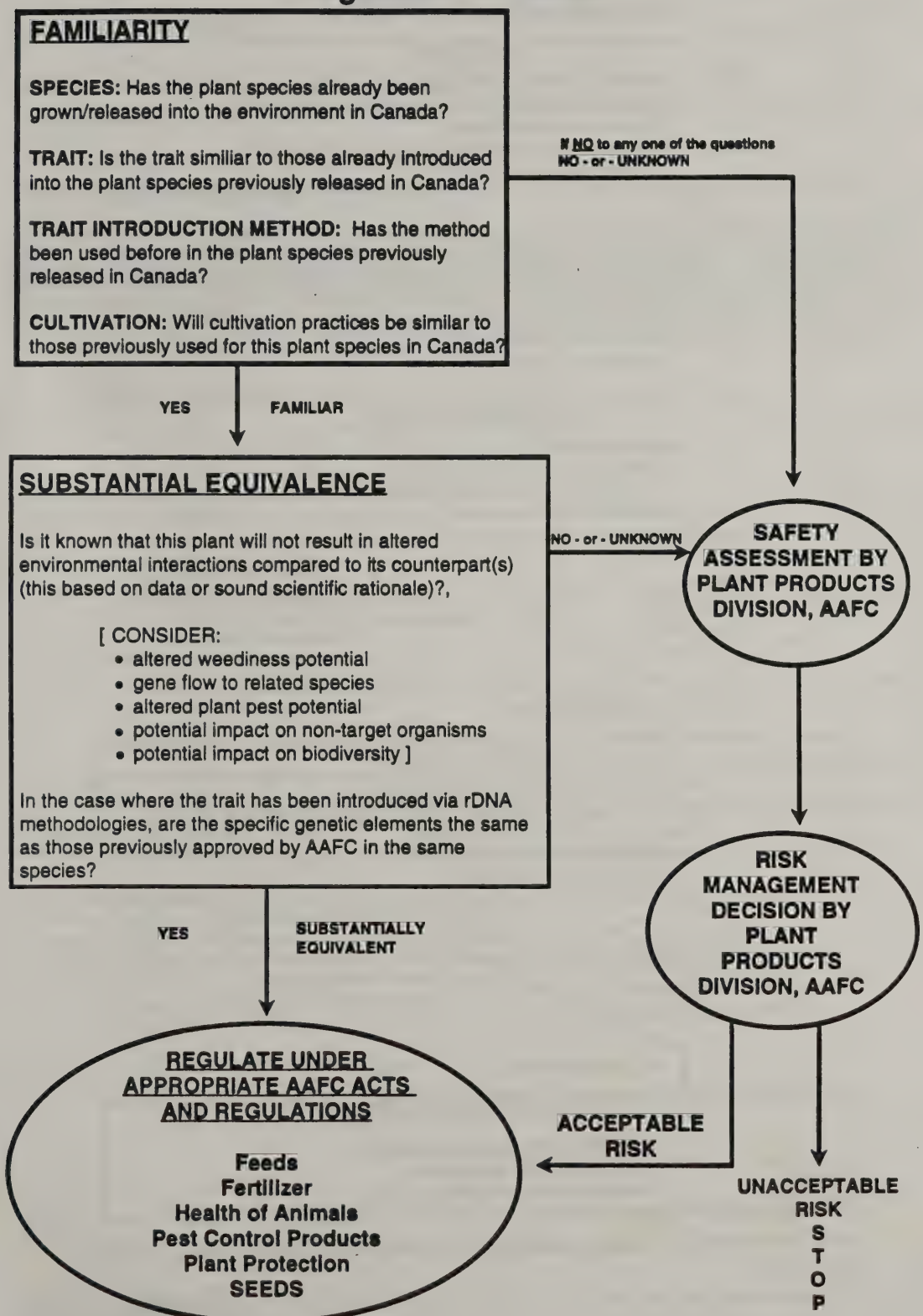
6) What deployment strategies are to be used (for example, in forestry, the avoidance of monocultures)?

D1.3 Potential environmental effects resulting from introgression.

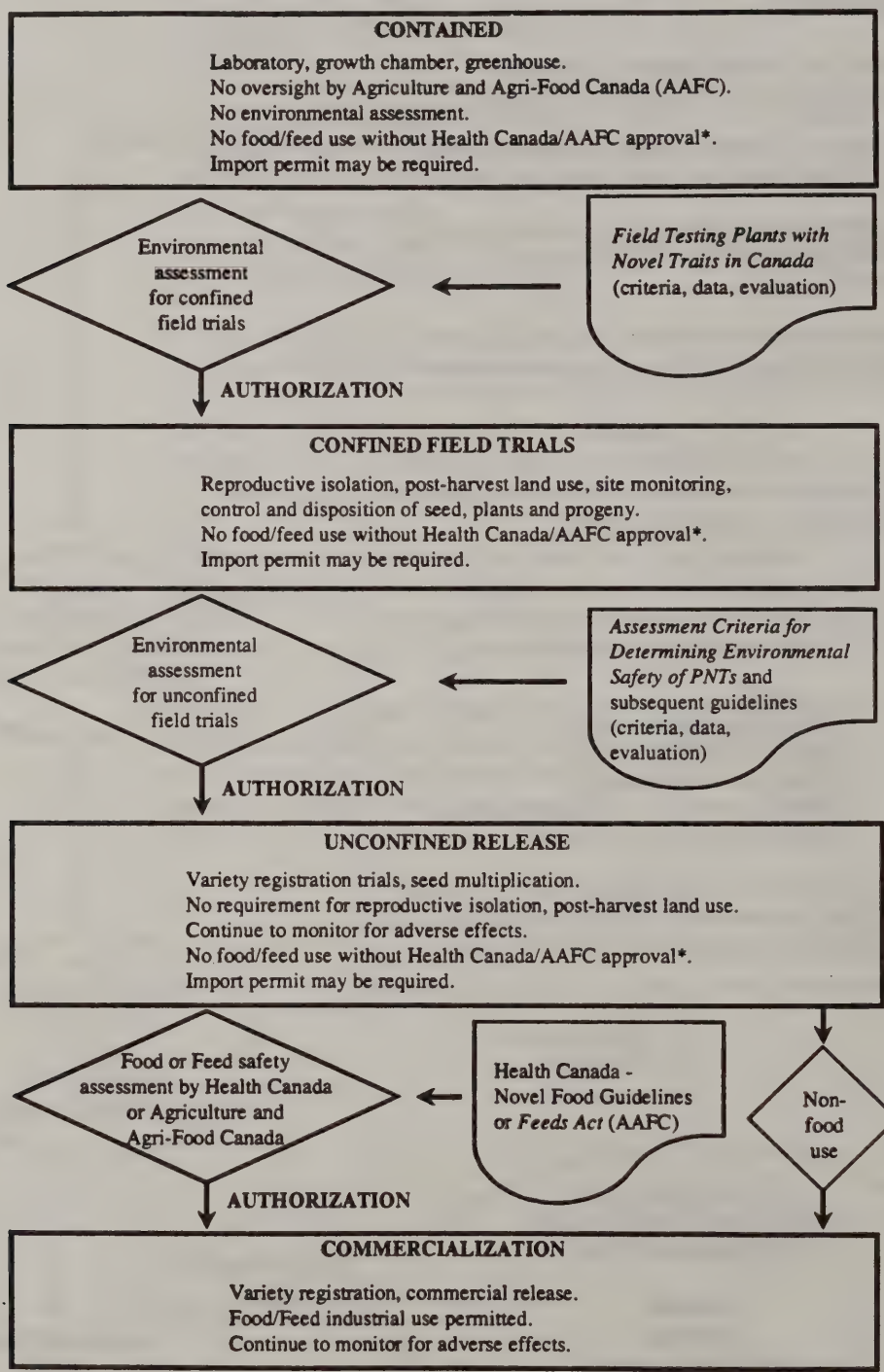
Where there is potential for gene flow from the PNT into related species, detail the consequences of novel gene introgression into those species and resulting expression. Interactions identified for the original PNT should be considered, as appropriate, for these species.

Appendix I - Figure 1

The Safety Based Model for the Regulation of Plants



Appendix I - Figure 2 Proposed regulatory steps in the risk assessment of PNTs by Agriculture and Agri-Food Canada.



* NOTE: Food and/or feed use must be obtained prior to commercialization, but may be sought at any stage of this process.

Appendix II - Table 1

DESCRIPTION OF THE PNT AND ITS MODIFICATION. Identity of PNT: _____(variety/genotype designation), a derivative of _____ (species).

Complete the following as appropriate[1]

Novel Gene Name (include source)[2]	Novel Gene Product[3]	Regulatory Sequences (include source)[4]	Other DNA Sequences[5]	Vector[6]

[1] If more than one novel gene is included in the PNT (including marker genes), provide information for each; separate non-linked genes with a blank row

[2] Provide the common name or symbol, including the biological source (or if synthesized, the source of the original gene code)

[3] Identify the gene product

[4] Provide the name and biological source of all regulatory sequences

[5] Provide the biological source of any other DNA sequences inserted

[6] Provide the biological source or common name of the transformation vectors (if applicable)

Appendix II - Table 2

THE NOVEL TRAITS. Identity of the novel traits expressed in the modified plant: _____ (variety/genotype designation), a derivative of _____ (species).

Gene Product (from Table 1)	Breakdown Products/By-products and Metabolic Pathways	Expression[1]	Activity of the Gene Product in the Plant[2]	Activity of the Gene Product in the Environment[3]

[1] Is the trait constitutively, tissue specific, or temporally expressed, or induced, and at what level?
[2] Do the gene products, by-products or breakdown products affect other metabolic pathways?
[3] Is there potential for the gene products, by-products or breakdown products to interact with other life forms?

Appendix II - Table 3

INTERACTIONS OF THE PNT. Relative phenotypic expression of _____ (variety/genotype designation), a derivative of _____ (plant species), relative to its counterpart.

Complete the following table as appropriate: (this information is to determine whether there are significantly different/altered interactions resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment). **In completing this table, applicants may consider it unnecessary or inappropriate to provide certain information. In these instances information requirements may be waived if valid scientific rationale is provided.**

Characteristic	Comparative Description[1]		Change[2]
	PNT	Counterpart	
Habit (annual, biennial, perennial)			
Vegetative vigour (biomass)			
Overwintering capacity (plant counts)			
Flowering period[3]			
Time to maturity[3]			
Seed Production[4]			
Dormancy[5]			
Reproductive Characteristics: • Outcross frequency within species (0-1, 2-20, 21-100%)			
• Cross Pollination vectors			
• Fertility - male			
• Fertility - female			
• Self compatibility			
• Asexual			

(continued on next page)

Appendix II - Table 3 (continued)

Stress Adaptations:			
Biotic[6]			
Abiotic			
Pesticide			
Residual Effects:[7]			
Composition:[8]			
Protein			
Lipid			
Others			
Endogenous Toxins (Define)			
Non-Endogenous Toxins:[9]			
Other Observations			

1 Include unit of measure, where appropriate.
2 Provide ratio, percentage change (for quantitative characteristics only) or visual description, where appropriate.
3 Approximate dates (month/day), and days from seeding.
4 Approximate yield (per ha) divided by seeding rate (per ha) for corresponding crop kind.
5 Determine viability before, and after set periods, of seeds buried in rot-resistant bags in the soil.
6 List life forms with which the PNT interacts differently from the unmodified plant or counterpart. Use the species-specific companion documents for guidance.
7 Include any observed residual effects on growth/development of any three of the five indicator species (forage grass or forage legume or annual cereal or corn or oilseed). See Appendix IV regarding conduct of residual effects trials.
8 Identify the major compositional components important in the commodity and any other observed composition changes.
9 Identify any introduced toxins and provide information on concentration, persistence and purpose.

Appendix II - Table 4

Summary of Anticipated Relative Impacts of Release of_____, a PNT of _____(species).

	Natural Ecosystem[1]				Managed Ecosystem[6]			
Effects of Release	**Degree of Change[2]**	**Geographic Scope[3]**	**Duration[4]**	**Relative Impact[5]**	**Degree of Change[2]**	**Geographic Scope[3]**	**Duration[4]**	**Relative Impact[5]**
Biodiversity: Plant populations								
Animal populations								
Microbe populations								
Substance presence/ persistence								
Sustainability[7]								
Agronomic-Silvicultural Practices[7]								
Resource conservation								
Other concerns (e.g., occupational health and safety)								
Overall Environmental Quality Changes								

(continued on next page)

Appendix II - Table 4 (continued)

1 Natural ecosystems are those subject to no, or minimal, human influence, eg., prairie grassland, forests, wetlands, rivers and alpine meadows
2 Degree of change: "+" if positive; "0" if no change;"-" if negative
3 Geographic scope: very local, local, national, continental, intercontinental
4 Duration: time in months/years (state which) of potential impact
5 Relative to the status quo, "+" if better; "0" if no change;"-" if worse
6 Managed ecosystems include farms and ranches, and areas subject to human influence, eg., gardens, roadside verges, urban parks, industrial and waste sites
7 Issues of sustainability and silvicultural practices need to be addressed for forestry species in both natural and managed ecosystems

Appendix III - Species Replacement/Competition Studies and Seed Dormancy Studies

These studies should be conducted in such a way as to determine whether the PNT has increased potential to become weedy in managed ecosystems or invasive of natural ecosystems.

Examples of replacement and seed dormancy studies may be found in Crawley et al., 1993, Linder and Schmitt, 1994, and in Rissler and Mellon, 1993.

Species Replacement

- Both the modified and an unmodified counterpart are studied.

- The two genotypes are studied in untended plots in the managed ecosystem, e.g., in field margins or in waste places, in different environments. These environments should include geographic locations where it is anticipated the modified plant will be released.

  (Note: replacement capacity studies should be conducted as confined field trials, and applications for authorization to field test genetically modified plants in these studies should be made to the Director of Plant Products using the latest version of the Directive *Field Testing Plants with Novel Traits (or Genetically Modified Plants) in Canada: Guidelines and Information Required for Submitting an Application for Field Trials Under Confined Conditions).*

- In year one, a known number of modified and unmodified seeds is sown randomly in replicated plots. Once scattered on the soil surface of the plots, there is no intervention. Plants are allowed to grow without addition of nutrients, the application of pesticides or the removal of other species by roguing. The percentage of seed emerging into seedlings and developing into flowering plants that eventually set seed should be recorded. At year end, seed is collected and viability of a subset determined. Seed is then returned to the plots from which it was harvested. Replacement values for modified and unmodified counterparts are determined (R = number of viable seeds harvested ÷ number of viable seeds sown).

- In year two, the process is repeated, and if necessary again in year three. There is no intervention with respect to soil preparation, pest control, etc.

- If the observed replacement value of the modified genotype is similar or lower than the unmodified genotype, or is less than 1 (a value less than 1 indicates the population will eventually decay to 0), it can be determined that the modified genotype is no more weedy nor invasive of natural habitats than is the unmodified genotype.

- Both the modified and an unmodified counterpart are studied.

Competition Studies

For an example of a competition study, see Fredshavn, J.R. and G.S. Poulsen (1993) "Growth behaviour and competitive ability of transgenic crops." Agric. Ecosystems Environ.

Seed Dormancy Studies

- Both the modified plant and an unmodified counterpart are studied.
- Bury in soil at different locations at varying depths a known number of seeds in non-degradable nylon mesh bags.
- Subsample from these at regular intervals and determine proportion of seeds that are dead, dormant and germinated.

Appendix IV - Residual Effects Bioassay

General Comments:

For Appendix II, Table 3, a field bioassay may be conducted to determine the potential residual effects of the PNT on interacting organisms.

Appendix II, Table 3 ("Interactions of the PNT") data should be collected concurrent with, or in confined field trials, including variety registration trials. Greenhouse experiments could also be considered.

Residual Effects Trials:

Applicants who have conducted confined field trials in the previous year on sites that require one or more years of post-harvest land use restriction are encouraged to consider using such sites for testing residual effects in the next season.

Figure 3. Example of sequence of trials over time and locations (sites)

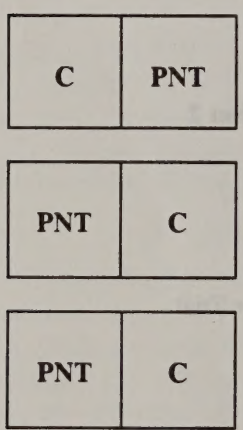
	Trial / Location 1	**Trial / Location 2**
Current Year	PNT trial	PNT trial
	↓	↓
Post Harvest Year 1	Residual Effects Trial + Significant	Residual Effects Trial - Significant
	↓	
Post Harvest Year 2	Residual Effects Trial	

The data collected for the residual effects trial could include: date of flowering, biomass production, concurrent soil moisture reading, nodulation studies (where applicable), and other relevant observable or measurable differences. A variety of plant species (see Appendix II, Table 3, footnote 7) common to that production area should be tested over the old (previous year's) PNT test site to test for species-specific effects. The conditions for the post-harvest land use would still have to be met (i.e., no canola following canola). If significant effects were detected the year following the trial (+ significant) then another year of testing at the same location would be warranted; such an outcome would be cause for further investigation of the nature of the environmental effect. Trials at a number of locations over one or two years are encouraged. Greenhouse assessments could also be considered for the assessment of residual effects, where appropriate.

Residual effects trials should follow sound experimental designs and statistical principles such as: randomization of treatments over experimental units, sufficient replication and testing of hypotheses using a commonly accepted level of significance (α=0.05). Control plots must be used in order to meaningfully test the significance of any residual effects of the PNT on subsequent plant species. The control plots are to be seeded over areas that were previously sown to the unmodified counterpart of the PNT in order to eliminate confounding effects of other crops or other cultural treatments such as fertilizer or herbicide applications. The layout of the residual effects trial should respect the layout of the previous PNT trial.

Figure 4. The following is an example of a suggested experimental layout of a residual effects trial. In the current year, the confined trial is laid out as a split-plot, in a randomized complete block design, with the main plots being the control (C) and the PNT; treatments (sub-plots) are randomized within these main plots. In post-harvest year 1, species plots are laid out perpendicular to the original main plots (i.e., in strips). **A.** Layout of the current year, confined trial. **B.** Layout of the residual effects trial; detail of one replicate (block).

A. Current Year Trial

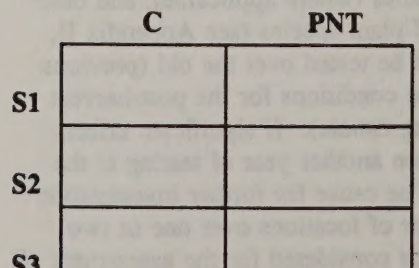


PNT = main plot, sown to plant with a novel trait.

C = main control plot, sown to unmodified counterpart

R1...R3 = replicates.

B. Residual Effects Trial



S1...S3 = species tested within residual effects trial.

PNT = main plot, previously sown to plant with a novel trai

C = main control plot, previously sown to unmodified counterpart.